An analysis of genetic population structure in red drum, *Sciaenops ocellatus*, based on mtDNA control region sequences

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Abstract.—Genetic information pertaining to stock structure in red drum (*Sciaenops ocellatus*) is equivocal, complicating attempts to develop sound fishery management and stock enhancement plans. In this study, genetic stock structure was examined by using mitochondrial DNA (mtDNA) control region sequences of 209 individual red drum from six locations in the Gulf of Mexico and five locations in the nearshore Atlantic Ocean off the southeastern United States. Eighty-one polymorphic sites within a 369 base-pair portion of the control region defined 134 different haplotypes which differed by up to 26 nucleotide substitutions. Red drum showed high average within-sample haplotype (0.98) and nucleotide (0.030) diversities. Sequence divergences between pairs of haplotypes ranged from 0.27% to 7.06% ($\bar{x}$=3.17%). Cluster analysis of haplotypes revealed very little phylogeographic structure among mtDNA lineages. However, a neighbor-joining tree based on nucleotide divergence between pairs of samples showed cohesion among Atlantic samples and, to a lesser degree, among Gulf samples. In contrast to a prior study, we found no evidence that red drum in Mosquito Lagoon, Florida, constitute a self-contained, reproductively isolated population. Hierarchical analysis of molecular variance supported the hypothesis that red drum are subdivided into two weakly diverged populations with a genetic transition in south Florida between Sarasota Bay and Mosquito Lagoon. This area forms a zone of differentiation between two genetically semi-isolated populations between which the structuring of heterogeneity differs from that under the assumption of panmixia. In addition, the analysis of molecular variance also indicated that red drum from Apalachicola Bay are genetically divergent from all other samples. The Atlantic and Gulf red drum populations are likely to respond independently to harvest regulations; these fisheries should continue to be managed separately. Additional subdivision of the Gulf stock between peninsular Florida and the northern and western Gulf may also be warranted.

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Red drum (*Sciaenops ocellatus*) is a pelagic marine fish that is distributed over a large geographic range that extends throughout the northern Gulf of Mexico and along the Atlantic coast of the southeastern United States to Cape Cod, Massachusetts (Ross et al., 1983). Juveniles grow rapidly in estuarine nurseries and reach reproductive maturity by age 4. At this age they join large schools of highly dispersing adults and for the remainder of their approximately 35-year life span (Murphy and Taylor, 1990), maintain a pelagic existence except to spawn during annual congregations at the mouths of bays and estuaries. The census size of the breeding population in the Gulf of Mexico has been estimated to be greater than 7 million individuals (Nichols[1]). Abundance in the Atlantic is thought to be of a similar magnitude (Gold et al., 1993).

Red drum supports highly valuable commercial and recreational fisheries throughout its range (Mercer, 1984). Fishing pressure is directed principally on subadult year classes (ages 2–4). A high rate of annual mortality among some cohorts (Murphy and Taylor, 1990) and an overall decline in abundance and recruitment during the 1980s (Goodyear[2]) have led to concerns regarding the status of red drum spawning stocks. Because there have been no prolonged offshore fisheries for adult red drum, biological and fishery-dependent data pertaining to their spawning stocks have been limited. Therefore, fishery managers have had to rely principally on virtual population analysis and simi-

[1] Nichols, S. 1988. An estimate of the size of the red drum spawning stock using mark/recapture. Southeast Fisheries Center, Natl. Mar. Fish. Serv., Pascagoula, MS.
[2] Goodyear, C. P. 1989. Status of the red drum stocks of the Gulf of Mexico. Report for 1989. Contract no. CRD 88/89-14. Coastal Resources Div., Miami Laboratory, Southeast Fisheries Center, Natl. Mar. Fish. Serv., Miami, FL.

lar analyses (e.g. Vaughan[3]) to manage the red drum fishery. Knowledge of stock structure in red drum, i.e. the geographic relation between spawning and recruitment, is needed to facilitate the management of this fishery.

Molecular studies have been used to define appropriate geographic scales for monitoring and managing exploited animal populations (Moritz, 1994), including marine fishes (e.g. Bentzen et al., 1996; Graves, 1996; Tringali and Bert, 1996). Although several population genetic studies have been conducted on red drum, the existing data are equivocal. On the basis of significant differences in allele frequencies at a single allozyme locus, Bohlmeyer and Gold (1991) concluded that red drum are weakly subdivided between the Atlantic Ocean (North Carolina and South Carolina) and the Gulf of Mexico. However, data from other allozyme surveys (Ramsey and Wakeman, 1987; Campton[4]) did not permit the rejection of the null hypothesis that red drum form a single panmictic gene pool. In contrast, on the basis of small but statistically significant differences in the frequencies of several composite mitochondrial DNA (mtDNA) haplotypes between red drum collected from North Carolina and South Carolina waters and red drum collected from the Gulf of Mexico, Gold and Richardson (1991) and Gold et al. (1993, 1994) reasserted that red drum are weakly subdivided between these regions, ostensibly along the south Florida coast. In addition, Gold et al. (1993) reported a pattern of mtDNA differentiation in Gulf red drum consistent with the isolation by distance model (Wright, 1943) for samples ranging from Florida to Texas. However, samples from a significant portion of the species range, including locations near the putative Atlantic-Gulf division (i.e. the eastern Florida seaboard), were not assayed in any of the above studies. An equally tenable but untested hypothesis is that isolation by distance occurs over the entire range of the species, perhaps in the absence of a genetic break at a particular geographic location.

Localized population subdivision in red drum has also been postulated. From comparisons with samples from North Carolina and South Carolina waters and samples from the Gulf of Mexico, Gold and Richardson (1994) proposed that red drum inhabiting Mosquito Lagoon, Florida, form a genetically distinct population. Red drum in Mosquito Lagoon reportedly have a life history uncharacteristic of other red drum. Adults occupy this coastal lagoon throughout the year and may complete their life cycle within the lagoon (Johnson and Funicelli, 1991). Adult red drum also occur throughout the year in other coastal lagoons adjacent to Mosquito Lagoon (e.g. Banana, Indian, and Halifax Rivers), but these have not been surveyed genetically. Rather than forming a self-contained population, red drum from Mosquito Lagoon may belong to a larger subpopulation occupying Florida Atlantic waters.

Owing to the perceived decline of red drum abundance in the 1980s, state agencies in Alabama, Florida, South Carolina, and Texas studied the feasibility of stock enhancement as a means of supplementing wild populations. Hatcheries in Florida, South Carolina, and Texas currently employ stocking on a large scale (McEachron et al., 1995; FDEP[5]). Hatchery programs potentially affect the gene pools of indigenous red drum populations by way of an inappropriate introduction of non-native individuals (Hindar et al., 1991) and by hatchery-induced inbreeding effects (Tringali and Bert, 1998). For example, because broodstocks for large-scale stock enhancement programs along the Atlantic seaboard have been obtained from Mosquito Lagoon and nearby estuaries (Halstead[6]), there is a potential for artificial genetic exchange between putatively separate gene pools (e.g. those of Mosquito Lagoon and the Carolinas).

State and regional fishery managers (Vaughan[3]; FDEP[5]) and hatchery managers (FDEP[7]) have adopted the stock structure scenario proposed by Gold et al. (1993) and Gold and Richardson (1994) in which red drum are divided into Gulf of Mexico and Atlantic populations, and those fish in Mosquito Lagoon comprise a unique, self-contained Atlantic subpopulation. However, several important questions regarding the genetic structure of red drum remain unanswered; each has serious implications for fishery management and stock enhancement programs. First, are red drum populations in the Gulf of Mexico and Atlantic really subdivided or do the observed genetic differences solely reflect isolation by distance over the range of the species? Second, if a genetic break does exist somewhere between the Gulf and the coast of the Carolinas, where is it?

[3] Vaughan, D. S. 1995. Status of the red drum stock on the Atlantic coast: stock assessment report for 1995. Southeast Fisheries Science Center, Natl. Mar. Fish. Serv., Beaufort, NC, 50 p.

[4] Campton, D. E. 1992. Gene flow estimation and population structure of red drum (*Sciaenops ocellatus*) in Florida. Final report, cooperative agreement no. 14-16-009-1522, National Fisheries Research Center, U.S. Fish and Wildlife Serv., Gainesville, FL.

[5] FDEP (Florida Department of Environmental Protection). 1993. A stock assessment of red drum (*Sciaenops ocellatus*) in Florida.Florida Marine Research Inst., Dep. Natural Resources, 100 Eighth Ave. SE, St. Petersburg, FL, 24 p.

[6] Halstead, B. 1997. Stock Enhancement Research Facility, Florida Department of Environmental Protection, 14495 Harlee Road, Port Manatee, FL 34221. Personal commun.

[7] FDEP (Florida Department of Environmental Protection). 1993. Marine fish stock enhancement and hatchery executive summary. Report to the legislature. FDEP, St. Petersburg, FL, 17 p.

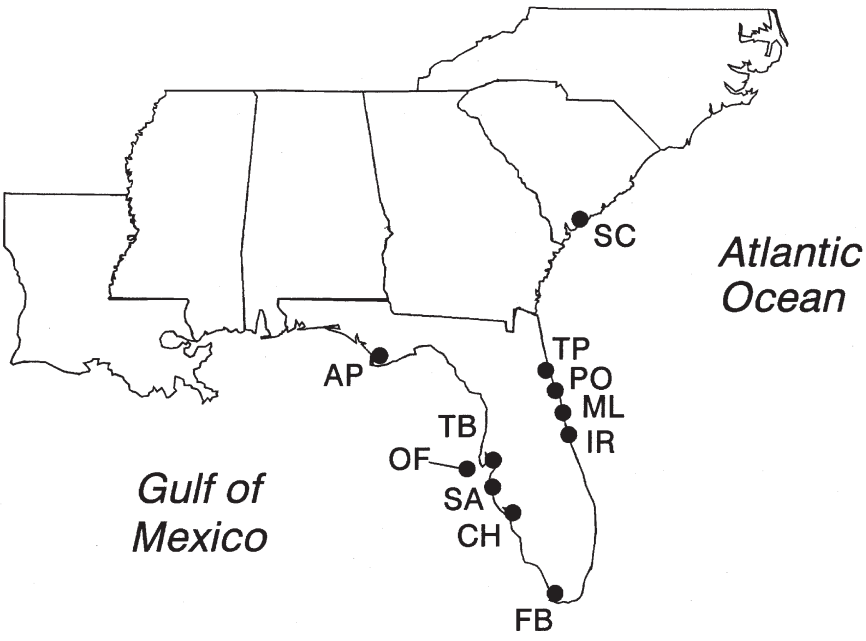

Figure 1

Sampling sites for red drum in waters off the southeastern United States. Florida: AP = Apalachicola Bay; TB = Tampa Bay; OF = offshore, Tampa Bay; SA = Sarasota Bay; CH = Charlotte Harbor; FB = Florida Bay; IR = Indian River; ML = Mosquito Lagoon; PO = Ponce de Leon inlet; TP = Tomoka Basin. South Carolina: SC = Charleston Harbor.

Third, are red drum in Mosquito Lagoon reproductively and genetically distinct or do they belong to a larger and heretofore unsampled east Florida population? To examine these questions, we obtained sequence data from the rapidly mutating mtDNA control region. Sequencing the control region has proven useful in intraspecific phylogeographic and population genetic studies of fishes (e.g. Fajen and Breden, 1992; Brown et al., 1993; Stepien, 1995; Stabile et al., 1996). We employed sampling and analytical regimes designed to test the various competing hypotheses of red drum population structure. In addition, by gathering baseline data for mtDNA control region diversity in red drum populations, we explored the potential for using the control region as a marker to assess and monitor ongoing stocking programs for wild red drum populations.

Materials and methods

Sample collection and DNA purification

Samples of red drum were collected with hook-and-line gear, trammel nets, and purse seines from riverine, estuarine, and offshore waters of the South Carolina coast (one location) and the east coast of Florida (four locations, sampled prior to stock enhancement activities), referred to collectively as the Atlantic samples; and the west coast of Florida (six locations), referred to collectively as the Gulf samples (Fig. 1). All specimens were collected between February 1992 and February 1997. Somatic muscle and liver tissue were dissected from each individual. Total length (range 280–1070 mm) of each individual was recorded prior to dissection. Tissues were frozen in liquid nitrogen and stored at –80°C in the laboratory until processing.

Approximately 100–400 milligrams of muscle or liver tissue were digested in 900 microliters of lysis buffer (0.1M Tris, pH 8.0, 0.05M ethylenediaminetetraacetid acid (EDTA), 0.2M NaCl, 1% weight by volume of sodium dodecyl sulfate (SDS), containing 1–2 milligrams of proteinase K) with moderate shaking for 3–5 h at room temperature. Following the addition of 150 microliters of chilled 8M potassium acetate, the SDS and cellular debris were precipitated for 30 min at 4°C and removed by centrifugation. Total genomic DNA was purified by phenol/chloroform extraction (Sambrook et al., 1989).

The DNA was concentrated by isopropanol precipitation, resuspended in 75 microliters of sterile water, and stored at –20°C.

mtDNA control region sequencing

Initially, we used the polymerase chain reaction (PCR; Saiki et al., 1988) and published primer sequences L15926 (Kocher et al., 1989) and H16498 (Meyer et al., 1990) to amplify a portion of the mtDNA control region of red drum. Double-stranded PCR was performed with Perkin Elmer AmpliTaq in a 50-µL reaction volume for 32 cycles in a DeltaCycler II System (Ericomp Inc., San Diego, CA), according to the methods described by Kocher et al. (1989). We amplified a 455-base-pair (bp) fragment of the control region for several individuals of red drum. However, because direct sequencing of the amplicon with the same PCR primers in the sequencing reaction yielded unsatisfactory results, we used a process of cloning and sequencing to design specific primers for red drum.

The 455-bp amplicon was cloned in pBluescript by TA cloning (Marchuk et al., 1990). Following denaturation of the plasmid DNA (Hattori and Sakaki, 1986), sequencing was done from both directions by the dideoxy termination method (Sanger et al., 1977) by using Sequenase version 2.0 (U.S. Biochemicals, Cleveland, OH) and [α-S^{35}] dATP (Dupont Biotechnology Systems, Wilmington, DE). Products of the sequencing reactions were resolved in 6% polyacrylamide/7-M urea gels that were vacuum dried at 80°C and autoradiographed with Kodak X-Omat AR film. We used the ESEE program (Cabot and Beckenbach, 1989) to align sequences. From these sequences, (Genbank accession no. AF054671), highly specific internal primers were designed for the control region of red drum. These primers partially overlapped the initial primers and were designated L15943 (5'-GTA AACCGGATGTCGGGGGTTAG-3') and H16484 (5'-GGAACCAGATACCAGGAATAGTTCA-3').

We used these custom primers to amplify a portion of the control region for 209 individuals in 50-µL reaction volumes. The PCR products were run on 1.2% low-EEO (Fisher Scientific, Norcross, GA) agarose gel during electrophoresis. The resulting bands were excised and then purified with GeneClean (Bio 101, La Jolla, CA). Double-stranded sequencing was conducted as described by O'Foighil et al. (1996).

Data analyses

Base composition, number of transitions (TSs), and number of transversions (TVs) were determined by using MEGA 1.01 (Kumar et al., 1993). Further analysis of base substitutions was conducted as in Brown and Clegg (1983). Each different haplotype was assigned a number, and the distribution of the different haplotypes was determined for each sample.

We used MEGA to generate a pairwise matrix of sequence divergence values between pairs of haplotypes and to construct an unrooted neighbor-joining tree; 200 replicates were used to estimate bootstrap values for the nodes. Sequence divergences were computed by using the pairwise-deletion option in MEGA; this distance estimator excludes sites at which indels occur on a pairwise basis. Haplotype and nucleotide diversity within samples and nucleotide divergence (D) between pairs of samples were estimated according to Nei and Tajima (1981) and Nei (1987) by using the DA option of REAP 4.0 (McElroy et al., 1992). The nucleotide divergence values were clustered by using the NJTREE program (Jin and Ferguson, 1990) based on the neighbor-joining method of Saitou and Nei (1987).

Geographic structuring of molecular variance among samples was examined by using the matrix of sequence divergences between all pairs of haplotypes in AMOVA 1.55 (Excoffier et al., 1992). In this analysis, the haplotype correlations (ϕ statistics) and their variance components were estimated in a hierarchical fashion: between regions, among samples within a region, and among individuals within samples. Statistical significances of ϕ values were computed by performing randomization tests with 500 replicates. Gold et al. (1993) concluded that red drum was subdivided into Atlantic and Gulf of Mexico populations. To determine the validity of this conclusion, we examined the spatial partitioning of molecular variance as follows. The between-region component of variance and ϕ_{CT} was first calculated for red drum samples divided into Atlantic (SC, TP, PO, ML, and IR) and Gulf (FB, CH, SA, OF, TB, and AP) regions. The compositions of the two groups were then adjusted by sequentially adding Atlantic samples to the Gulf group and then sequentially adding Gulf samples to the Atlantic group. After each addition, the apportioning of molecular variance between the resulting groups was recalculated. For example, IR (the Atlantic sample closest to the Gulf) was added to the Gulf group and tested against the remaining Atlantic samples (ML, PO, TP, and SC). ML (the second closest sample) was then added to the Gulf-plus-IR group; that grouping was then tested against PO, TP, and SC. This process was repeated until only a single sample remained in one group.

Finally, to test for an association between interpopulation D values and geographic distance (isolation-by-distance), we performed the Mantel test (BIOMstat, version 3.0; Rohlf and Slice, 1995) for samples grouped

by region (Atlantic or Gulf) and for all samples combined. The statistical significance of the association was tested by random permutation analysis by using 500 replicates (Sokal and Rohlf, 1995).

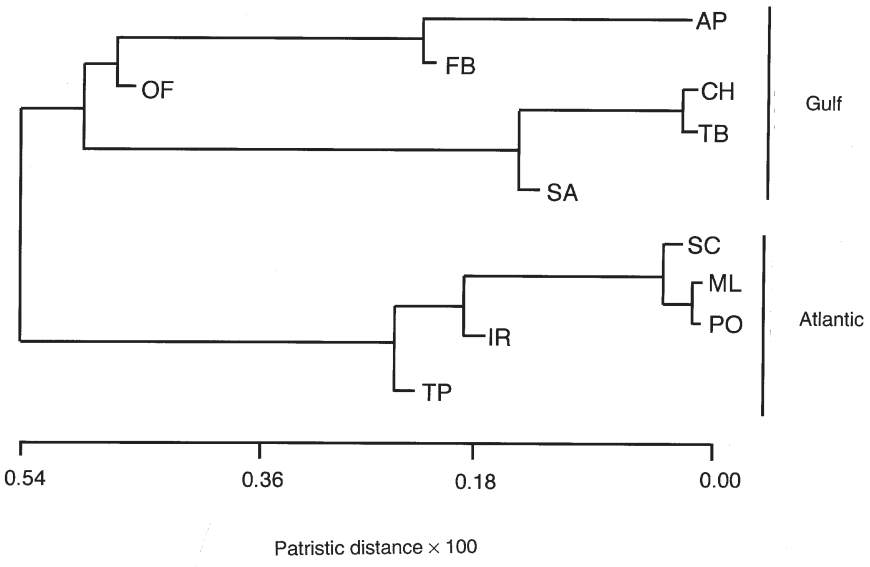

Figure 2

Neighbor-joining tree based on mtDNA nucleotide divergence between samples of red drum from Florida and South Carolina nearshore waters. Sample location and abbreviates are defined in Figure 1.

Results

We analyzed sequence data from a 369-bp portion of the mtDNA control region for 209 red drum. A total of 81 polymorphic sites were observed among all individuals. Of these, 67 sites had single-state, 11 had double-state, and 3 had triple-state transformations, totaling 98 polymorphisms and including two indels. The first indel consisted of an insertion of a pyrimidine (T or C) at position 160 and occurred in 10 individuals; the second indel consisted of a deletion of a purine (A) at position 210 and occurred in one of these individuals (Table 1). Seventy-three of the substitutions were TSs and 22 were TVs. As with control regions of other fishes (Stepien, 1995), the relative frequencies of the four nucleotide bases differed; adenine was the most prevalent (39%), followed by thymine (27%), cytosine (23%), and guanine (11%). The TS:TV ratio was 3.4:1 and was similar to ratios reported for marine and freshwater fishes (Stepien, 1995; but see Brown et al., 1993).

We observed 134 different haplotypes in the 209 individuals sequenced (Table 1). Sequences of these haplotypes have been deposited in GenBank under the accession numbers AF054672–F054805. Twenty-nine haplotypes were shared by more than one individual. Two haplotypes, no. 56 and no. 83, were shared by nine and nineteen individuals, respectively, which were widely dispersed among seven samples. Twenty-six haplotypes occurred infrequently, 25 in two to five individuals scattered among two to five samples, and one in two members of a single sample. Of the 19 individuals with haplotype no. 83, 17 were from the Atlantic and two from the Gulf. The percentage of different haplotypes in any one sample varied from 76% to 100% ($\bar{x}$=87%). Haplotype diversity within samples ranged from 0.95 to 1.00 ($\bar{x}$=0.98, SE=0.00) and nucleotide diversity ranged from 0.025 to 0.037 ($\bar{x}$=0.030, SE=0.003).

Percent sequence divergences between pairs of different haplotypes ranged from 0.3% to 7.1% ($\bar{x}$= 3.2%; SE=0.017). Between any two different haplotypes, the number of nucleotide differences varied from one to 26 ($\bar{x}$=12). The topology of the unrooted tree neighbor-joining (not shown) revealed that the 134 haplotypes were not phylogeographically structured. Haplotypes observed in Gulf and Atlantic samples were scattered throughout the tree; with the exception of two terminal groupings, nodes on the tree had low statistical support. Internal branch lengths of the tree were generally short; however, one interior branch was relatively long, and it defined the only well-supported major clade (bootstrap value=85). This clade consisted of 23 haplotypes, including the 10 haplotypes that had the insertion at position 160. The 10 insertion-bearing haplotypes were found in nine Atlantic individuals but in only one Gulf individual.

The D values between pairs of samples ranged from –0.08% to 0.10%. In the neighbor-joining cluster analysis, cohesion of the samples within geographic regions was generally observed (Fig. 2). All Atlantic samples formed a distinct clade which was separated by the longest branch of the tree from the clade formed by the Gulf samples. Less cohesion was observed among the Gulf samples, although the geographically proximal SA, TB, and CH samples clustered closely together.

Table 1

Distribution of red drum mitochondrial DNA control region haplotypes from 11 locations. Abbreviations for sample locations are defined in Figure 1.

	Gulf						Atlantic				
Haplotype	AP	TB	OF	SA	CH	FB	IR	ML	PO	TP	SC
1	1										
2	3					1					
3	1						1		1	1	
4	1										
5	1										
6	1								1		
7	1		1		1	1			1		
8	1										
9	1										
10	1										
11	1										
12	1										
13	1										
14	1										
15	1										
16	1					1					
17	1										
18	1										
19		1									
20		1									
21[1]		1									
22		1									
23		1									
24		1									
25		2									
26		1									
27		1									
28		1									
29		1									
30		1									
31		1									
32			1							1	
33			1								1
34			1								
35			1								
36			1								
37		1	1								
38			1								
39			1								
40			1								
41			1								
42				1							
43				1							
44				1							
45				2							
46				1							
47				1							
48				1							
49					1						
50					1						
51					1						
52					1						
53					1	2			1	1	
54					1						

continued

Table 1 (continued)

	Gulf						Atlantic				
Haplotype	AP	TB	OF	SA	CH	FB	IR	ML	PO	TP	SC
55		3			3	1					
56		2			1	1	1	1		2	1
57		1			1						
58					1			1			
59					1						
60					1						
61					1					1	
62					1						
63			1		1						
64					1						
65					1						
66						1					
67						1					
68						1					
69						1					
70						1					
71						1					
72			1			1			1		
73						1			1		
74						1					
75						1					
76						1					
77						1					
78						1					
79							1				
80							2		1		1
81							2				1
82							1				
83		1	1				3	5	3	3	3
84[1]							1			1	
85							1				
86							1		1	1	1
87							1				
88							1				
89[1]							1				
90								1			
91								1			
92								1			
93[1]								1			
94								1			
95[1]								1			
96								1			
97								1			
98								1			
99								2			
100								1			
101[1]								1			
102								1			
103									1		1
104									1		
105									2		2
106									1		
107									1		
108									1		
109									1		
110									1		

continued

Table 1 (continued)

	Gulf						Atlantic				
Haplotype	AP	TB	OF	SA	CH	FB	IR	ML	PO	TP	SC
111									1		
112									1		
113										1	
114										1	
115										1	
116										1	
117[1,2]										1	
118										1	
119										1	
120										1	
121										1	
122[1]										1	
123										1	
124										1	1
125											1
126											1
127											1
128											1
129											1
130[1]											1
131											1
132											1
133											1
134											1
Total	20	22	14	8	20	20	17	21	22	23	22

[1] Haplotypes with an insertion at position 160.
[2] Haplotype with an insertion at position 210.

The D value between the ML sample and the remaining pooled Atlantic samples was –0.05%. In contrast, the D value between the ML sample and the Gulf samples ranged from –0.02% to 0.076%.

The analysis of molecular variance (AMOVA) for all samples yielded a ϕ_{ST} value of –0.001, indicating that no significant heterogeneity was detected between any two samples. For the geographic analysis, in which samples were divided into shifting regional subsets, the variance components among samples within groups and among individuals within samples were not significant for any grouping. Significant values of ϕ_{CT} were observed in five of the 10 groupings (Table 2). In four of the five significant groupings, the division occurred in peninsular south Florida. Overall, results of the AMOVA suggest that a genetic transition occurs in red drum along the Florida coast between Sarasota Bay in the Gulf and Mosquito Lagoon in the Atlantic. However, the highest ϕ_{CT} value and between-group variance component were observed when samples were grouped according to their actual Atlantic and Gulf locations (Table 2, grouping 1). The significant ϕ_{CT} value for the AP sample versus all other samples suggests that an additional genetic discontinuity occurs in Gulf waters off northwest Florida.

In the Mantel test between interpopulational D and geographic distance, no association was observed among the Atlantic samples (P=0.20) or among the Gulf samples (P=0.053). However, a significant association was observed for all red drum samples (P<0.01), reflecting the genetic transition that occurs in south Florida.

Discussion

Genetic population structure

Because there are few absolute barriers to gene flow in the ocean, it is generally expected that marine species with continuous distributions, large populations, and high levels of larval and adult dispersal should have very little intraspecific population structure over large geographic areas (Avise, 1987; Palumbi, 1992).

Table 2

Geographic analysis of molecular variance in the mtDNA control region of red drum. The table lists the ϕ_{CT} values and between-group variance components for the ten possible geographical groupings and the probability P of finding a more extreme variance component by chance (500 permutations). In each group, the letter G represents samples from the Gulf of Mexico (G_1=AP, G_2=TB, G_3=OF, G_4=SA, G_5=CH, G_6=FB) and the letter A represents samples from the Atlantic Ocean (A_1=IR, A_2= ML, A_3=PO, A_4=TP, A_5=SC). Abbreviations for sample locations are defined in Figure 1.

Grouping	First group	Second group	Variance between groups (%)	Φ_{CT}	P
1	$G_1G_2G_3G_4G_5G_6$	$A_1A_2A_3A_4A_5$	1.96	0.020	<0.002
2	$G_1G_2G_3G_4G_5G_6$ A_1	$A_2A_3A_4A_5$	1.40	0.014	0.012
3	$G_1G_2G_3G_4G_5G_6$ A_1A_2	$A_3A_4A_5$	0.39	0.004	NS
4	$G_1G_2G_3G_4G_5G_6A_1A_2A_3$	A_4A_5	–0.17	–0.002	NS
5	$G_1G_2G_3G_4G_5G_6A_1A_2A_3A_4$	A_5	–0.54	–0.005	NS
6	$G_1G_2G_3G_4G_5$	$G_6A_1A_2A_3A_4A_5$	1.48	0.015	0.004
7	$G_1G_2G_3G_4$	$G_5G_6A_1A_2A_3A_4A_5$	0.78	0.008	0.044
8	$G_1G_2G_3$	$G_4G_5G_6A_1A_2A_3A_4A_5$	0.73	0.007	NS
9	G_1G_2	$G_3G_4G_5G_6A_1A_2A_3A_4A_4$	0.74	0.007	NS
10	G_1	$G_2G_3G_4G_5G_6$ $A_1A_2A_3A_4A_5$	1.50	0.015	<0.002

Red drum is a pelagic marine fish with these demographic and life history characteristics. However, although some genetic exchange may occur between red drum from distant locations, populations differ from the expectation of genetic homogeneity. Hierarchical analysis of the structuring of genetic variance supports the hypothesis that the species is weakly subdivided between the Atlantic Ocean and the Gulf of Mexico. The existence of the subdivision is also supported by cluster analysis of sequence divergence values. The geographic coverage of our samples, particularly the inclusion of samples from Florida's eastern seaboard, allowed us to infer that the genetic break separating these two populations occurs in south Florida. Another genetic discontinuity apparently occurs in Gulf waters off northwest Florida.

The Atlantic-Gulf subdivision in red drum may result from a combination of extrinsic and behavioral factors. Gold et al. (1993, 1994) summarized a number of potentially important oceanographic and geographic factors. Because the southernmost portion of the east Florida shelf is extremely narrow and provides little of the neritic habitat (Jones et al., 1985) generally occupied by adult red drum, it may represent a significant barrier to adult migration. Biotic factors largely preclude large-scale passive dispersal of eggs and larvae (Peters and McMichael, 1987), and widespread dispersal at the juvenile stage is rare (Murphy and Taylor[8]). If partitioning of genetic variation in red drum results from adult migration or vagrant movement between spawning locations, it is not evident from studies of fish movement. Although very few tagged adult red drum have been recaptured after spending significant periods at large, the evidence suggests that some are highly mobile and may disperse to distances of up to 320 km (Woodward and Nicholson, 1997; Crabtree[9]). However, movement of red drum between the Atlantic and Gulf regions has not been documented. In apparent contrast to the mark-recapture data, recent ultrasonic tracking studies of adults provide limited evidence for spawning fidelity to certain Atlantic estuaries over two-to-three-year periods, and potentially longer (Nicholson and Jordan[10]). Overall, the available movement data for adult red drum are not adequate to draw conclusions relating to regional recruitment processes and patterns of dispersal. Nevertheless, it is clear from the genetic data that reproductive exchange between spawning populations in the Atlantic and Gulf regions is limited.

Because red drum from Mosquito Lagoon were used to produce hatchery populations for at least two stock enhancement programs along the Atlantic seaboard, it was important to determine whether the spawning aggregation within that system represented a self-contained, genetically divergent popu-

[8] Murphy, M. D., and R. G. Taylor. 1989. Tag/recapture and age validation of red drum in Florida. Final report, NOAA grant NA86-WC-H-06136, National Marine Fisheries Service, Pascagoula, MS, 27 p.

[9] Crabtree, R. 1997. Florida Marine Research Institute, Department of Environmental Protection, St. Petersburg, FL. Unpublished data.

[10] Nicholson, N., and S. R. Jordan. 1994. Biotelemetry study of red drum in Georgia, November 1989–June 1993. Coastal Resources Division, Georgia Department of Natural Resources, Brunswick, GA, 65 p.

lation. Contrary to the study of Gold et al. (1994), we found no evidence to support the hypothesis that red drum in Mosquito Lagoon are reproductively isolated from other Atlantic red drum. In our survey of the mtDNA control region, there was no nucleotide divergence between Mosquito Lagoon and other Atlantic samples, whereas divergence values between the Mosquito Lagoon and the Gulf samples were among the highest. Furthermore, other investigators also found no significant differences at the allozyme loci that putatively distinguish Mosquito Lagoon red drum from other red drum (Campton[4]; Crawford and Bert[11]). This lack of difference suggests that the allozyme frequency differences observed by Gold and Richardson (1994) may not be temporally stable. A lack of samples geographically proximal to Mosquito Lagoon may have also influenced the outcome of that study. Considering all the evidence, it seems more likely that red drum from Mosquito Lagoon belong to the larger genetic population occupying nearshore Atlantic waters of the southern United States. Although it is generally better to obtain hatchery broodstock from locations within or near the intended release site (Utter, 1998), the use of Mosquito Lagoon red drum as a source of broodstock in Atlantic coast stock enhancement programs should not produce a negative genetic impact on wild red drum.

Implications for fishery and hatchery management

Because red drum support valuable fisheries throughout the Gulf of Mexico and Atlantic seaboard, the species is of special concern to state and regional fishery management agencies. Our results support the hypothesis that a genetic transition in red drum population structure occurs in south Florida. In theory, it requires the regular exchange of only a few individuals between breeding populations to homogenize their genetic composition (Slatkin, 1987). Thus, genetic exchange between Atlantic and Gulf stocks by any recruitment process must be sufficiently low to allow genetic differences to accumulate or, if the differences reflect a historical disassociation, for them to be maintained. The two red drum stocks can best be described genetically as demes, separate semi-isolated groups between which the structuring of heterogeneity differs from the assumption of panmixia (Hartl and Clark, 1989). Therefore, the Atlantic and Gulf stocks are likely to respond independently to harvest regulations and these fisheries should continue to be managed separately.

[11] Crawford, C., and Bert, T. 1997. Florida Marine Research Institute, Department of Environmental Protection, St. Petersburg, FL. Unpublished data.

Gold et al. (1993) observed a pattern of isolation by distance in the distribution of mtDNA haplotypes among Gulf samples that ranged from the southeast coast of Texas to the southwest coast of Florida. Although we did not observe a similar pattern for Gulf samples ranging from Apalachicola Bay to Florida Bay, the probability value for the Mantel coefficient was nearly significant at the 0.05 level and the AMOVA value for the Apalachicola sample versus all other samples was highly significant. This indicates that the minimum geographic scale at which the isolation-by-distance mechanism operates is greater than the distance between Apalachicola Bay and Florida Bay (approximately 670 km) or that genetic discontinuity also exists between Florida Gulf red drum and red drum inhabiting the northern and western Gulf of Mexico. Accordingly, cooperative management of the Gulf fishery on a regional basis is appropriate. No pattern of isolation-by-distance was evident for red drum along the southern Atlantic seaboard; the fishery between South Carolina and southeast Florida should be managed as a single unit.

Our principal objective for undertaking this study was to improve upon available genetic information relating to red drum population structure for fishery management purposes. Our most informative statistical tools were those that assessed relationships among the samples. Genotype frequency differences accumulate quickly in subdivided populations compared with the rate at which distinct phyletic lineages emerge and sort geographically. Moreover, mitochondrial DNA restriction fragment length polymorphism and sequence data for marine populations are typically characterized by haplotype distributions which consist of a few numerically and geographically prevalent haplotypes and many rare, geographically restricted haplotypes that may be important with respect to population structure. Therefore, as our results and the results of Tringali and Bert (1996) demonstrate, genetically-based management units (*sensu* Moritz, 1994), especially in marine fishery stocks, may be more easily identified by applying population-level analyses that take full advantage of both differences in genotype frequencies among samples and phylogenetic relatedness of individual genotypes. Statistical tests of association when applied to skewed haplotype distributions often lack the power to detect the low levels of population divergence that may characterize marine populations. Moreover, these tests ignore the interrelatedness of haplotypes in terms of sequence similarity or difference. The two principal analytical methods we employed, clustering of intersample nucleotide divergence values and AMOVA, are based on both the occurrence of haplotypes at particular locations and their sequence similarity to other

haplotypes at those locations. This approach may be particularly important in studies of pelagic marine species because, like red drum, genetic divergence separating populations is often buried within a high background of overall genetic diversity.

Finally, Moritz (1994) described the potential of using mtDNA as a marker for evaluating the success of stock enhancement programs. For this application, the mtDNA haplotypes borne by hatchery fish should be sufficiently rare in wild populations. The portion of control region we examined provides an excellent source of naturally occurring genetic markers. Because the percentage of wild red drum individuals with different haplotypes in any given sample averaged 87% and ranged up to 100%, a genetic monitoring program using this mtDNA fragment to track haplotypes borne by hatchery-released red drum after release should allow for assessment of the survival and reproductive output of these fish in the natural environment. In addition, nucleotide substitutions in this portion of the control region could be used to estimate the contribution of each female parent to the broods. This information will be important in the evaluation of breeding protocols designed to optimize levels of genetic variability in hatchery broods, in the assessment of genetic risk posed to wild red drum populations by hatchery stocking programs (e.g. Tringali and Bert, 1998), and in the evaluation of stock supplementation programs.

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